# X Jornadas Olfativas

# Table of contents

# Transcriptional adaptation of olfactory sensory neurons to GPCR identity and activity

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**Code:** 2
**Type of abstract:** TALK

In mammals, chemoperception relies on a diverse set of neuronal sensors able to detect chemicals present in the environment, and to adapt to various levels of stimulation. The contribution of endogenous and external factors to these neuronal identities remains to be determined. Taking advantage of the parallel coding lines present in the olfactory system, we explored the potential variations of neuronal identities before and after olfactory experience. We found that at rest, the transcriptomic profiles of mouse olfactory sensory neuron populations are already divergent, specific to the olfactory receptor they express, and are associated with the sequence of these latter. These divergent profiles further evolve in response to the environment, as odorant exposure leads to reprogramming via the modulation of transcription. These findings highlight a broad range of sensory neuron identities that are present at rest and that adapt to the experience of the individual, thus adding to the complexity and flexibility of sensory coding.

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[1] Transcriptional adaptation of olfactory sensory neurons to GPCR identity and activity. Nature communications. 2022

# Exploring the molecular basis of sensory deficits in DWV-A infected honeybees

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**Code:** 3
**Type of abstract:** TALK
**Keywords:** Synaptic Homeostasis, Olfactory Kinetics, Electrophysiology, RNAi, honeybees

Deformed Wing Virus type A (DWV-A) is a neurotropic pathogen that severely impacts the neurophysiology of the honeybee (*Apis mellifera* L.), altering its olfactory sensitivity. Despite its importance, the molecular mechanisms underlying these deficits in this non-canonical model remain poorly explored. This study integrates a transcriptomic and functional approach to investigate the basis of such sensory alterations. Through RNA-seq analysis of heads from infected bees, we identified 147 differentially expressed genes, showing a marked modulation in cell communication processes and transmembrane transport. From this analysis, we selected a triad of genes *Neto, Eaat-2*, and a Kainate-type receptor whose orthologs in vertebrate models are critical components in regulating synaptic architecture and homeostasis, particularly associated with the glutamatergic axis. RT-qPCR analysis revealed that DWV-A alters the expression dynamics of these genes in a tissue-specific manner, suggesting a compromise of synaptic regulation at both central and peripheral levels. To validate their functional relevance, we employed RNA interference (RNAi) and electroantennography (EAG). Silencing these genes mimicked the defective peripheral reception symptoms observed in infected bees, significantly reducing response amplitude to floral volatiles. Notably, *Neto* deficiency increased activation latency (Time to Peak), while *Eaat-2* reduction prolonged recovery time and decreased the repolarization slope. These findings suggest that the functional integrity of this conserved axis is fundamental for sensory signal kinetics. The disruption of these homeostatic nodes by DWV-A provides a molecular framework for understanding sensory deterioration in the colony, highlighting the relevance of investigating synaptic plasticity mechanisms in social insects.

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# Perineurial glia mediates the regulation of olfactory reception via Eaat2

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**Code:** 4
**Type of abstract:** TALK
**Keywords:** Perineurial glia Eaat2 Olfactory receptor neurons (ORNs) Drosophila melanogaster Neuron-glia interaction

Glial cells are closely associated with neurons throughout the nervous system. Historically, they have been attributed a primarily supportive role, contributing to the maintenance of neuronal activity and homeostasis. However, there is growing evidence of the regulatory role of glia in the nervous system. Although various molecular mechanisms mediating neuron-glia interaction have been described in the central nervous system (CNS), these processes remain less characterized in the peripheral nervous system (PNS).

The olfactory system of Drosophila melanogaster is a relatively simple and genetically manipulable model for studying neuron-glia interactions in the PNS. Previous studies have shown that the activity of olfactory receptor neurons (ORNs) can modulate the response of perineurial glial cells in the antenna marked by the Mz317-Gal4 line. Furthermore, it has been observed that silencing the expression of the Eaat2 gene in these glial cells which encodes a transmembrane protein involved in the transport of taurine and aspartate affects behavior guided by olfactory stimuli.

In this study, we analyzed the modulatory role of Eaat2 in Mz317 glial cells on ORN activity. Our results show that intense odor stimulation causes changes in the expression levels of Eaat2 in Mz317 glia. Using a combination of calcium imaging and RNA interference (RNAi), we also demonstrate that inhibition of Eaat2 in these glial cells modifies the response of ORNs to odors, without altering the morphology of Mz317 glia. Together, these data indicate that Eaat2 in Mz317 glia plays a key role in modulating ORN responses to odor stimuli, highlighting a regulatory function of glia in peripheral olfactory processing.

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# A single nuclei atlas of the rabbit Olfactory Bulb

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**Code:** 5
**Type of abstract:** TALK
**Keywords:** Rabbit, transcriptomic profiling, olfactory bulb, odour procesing,

The olfactory bulb (OB) of the rabbit is the primary centre for olfactory processing, yet its cellular organisation at transcriptomic resolution has remained unexplored. In this study, we generated the first single-nucleus RNA-sequencing (snRNA-seq) atlas of the rabbit OB using eight samples from adult and juvenile males and females. Nuclei were fixed and barcoded using Evercode WT v3 (Parse Biosciences), and snRNA-seq libraries were sequenced at Novogene (UK) on an Illumina NovaSeq X Plus platform to obtain 150 bp paired-end reads. FASTQ files were initially processed with the Parse Biosciences pipeline, which performed demultiplexing, alignment to the Oryctolagus cuniculus reference genome, and generation of quality metrics. The resulting count_matrix.mtx, cell_metadata.csv, and all_genes.csv files were then downloaded and analysed in RStudio, where data normalisation, integration, clustering, and dimensionality reduction were carried out. After quality control and integration of all samples, we identified 33 transcriptionally distinct clusters (00–32). These clusters encompassed the major cellular lineages of the OB, including glutamatergic projection neurons (mitral-like, tufted, and AOB-like), GABAergic interneurons, immature DCX-positive neurons, central glia (astrocytes, oligodendrocytes, and microglia), peripheral glia associated with nerve fibres, vascular populations, and multiciliated ependymal cells. The UMAP structure revealed clear segregation between excitatory projection neurons, inhibitory interneurons, and glial lineages, while immature neuronal populations formed a continuous gradient consistent with active neurogenesis. This atlas provides the first high-resolution molecular reference of the rabbit olfactory bulb, offering a robust framework for investigating neurogenesis, olfactory circuit organisation, and developmental and sex-related variation in lagomorphs.

# Neuronal network organization and coding in the olfactory system of amphibians

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**Code:** 6

**Type of abstract:** PLENARY LECTURE

**Keywords:** vertebrate olfaction; olfactory bulb circuitry; amphibians; odor processing; Xenopus laevis; comparative neuroanatomy

Vertebrate olfactory systems are often interpreted through principles derived mainly from rodents, including the idea that each olfactory receptor neuron sends a single, unbranched axon to one defined target in the olfactory bulb. Comparative evidence challenges this view. In amphibians, olfactory receptor neuron axons frequently bifurcate and distribute input more broadly. In addition, projection neurons in amphibians, teleost fishes, and reptiles often show complex dendritic morphologies, suggesting that olfactory bulb circuitry varies substantially across vertebrate groups.

These findings indicate that the organization of vertebrate olfactory circuits is more diverse than commonly assumed. The atypical organization observed in amphibians may underlie alternative mechanisms of olfactory processing. Functional studies in larvae of the African clawed frog (Xenopus laevis) support this view and suggest that similar processing differences may exist more broadly among amphibians. Understanding vertebrate olfaction therefore requires a broader comparative approach across major vertebrate lineages, complementing the insights gained from rodent models.

# Integration of main and accessory olfactory pathways

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**Code:** 7
**Type of abstract:** PLENARY LECTURE

At least two parallel sensory systems mediate olfaction in most mammals: the main and accessory olfactory systems. Specialized peripheral sensory organs detect chemosensory cues and relay them separately to the main and accessory olfactory bulbs, where olfactory information is processed in parallel. Here, we investigate downstream circuits and ask whether they integrate main and accessory olfactory information. First, we trace axonal projections of main and accessory olfactory bulb principal neurons. To this end, we combine stereotactic microinjections with targeted expression of recombinant adeno-associated viral genomes to identify downstream target regions of both pathways. We identify two amygdaloid nuclei as shared projection targets of the main and accessory olfactory pathways. Second, we employ single-cell patch-clamp recordings in acute brain slices to describe the physiology and morphology of neurons constituting these putative integration areas. By quantifying electrophysiological and morphological features, we reveal distinct cell types in both nuclei. Third, we dissect functional connectivity between main and accessory olfactory bulb projections and these regions. To this end, we combine optogenetic activation of axonal terminals with single-cell patch-clamp recordings. Together, we describe both the anatomical and functional convergence of main and accessory olfactory pathways in the amygdala. Moreover, we extend these findings by systematically describing cell types and connectivity in both nuclei based on electrophysiological and morphological characteristics.

# G-proteins modification in vomeronasal organ alteration in mice and farm animals

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**Code:** 8
**Type of abstract:** TALK
**Keywords:** chemical communication, farm animals, G-proteins, mouse, pathology, vomeronasal organ

G-proteins play a key-role in vomeronasal organ (VNO) function and, therefore, in mammals chemical communication. In particular, the Gαi2 protein is coupled to the vomeronasal type 1 receptors (V1Rs), while the Gαo to the V2Rs, both playing a pivotal role in vomeronasal sensory transduction. Previous studies showed that the genetic deletion of these proteins has been associated with several alterations in social and reproductive behavior in mice. Recently, we have described that the VNO can be affected by natural and pathological changes influencing animal behavior in cats, pigs and mice. This communication aims to describe how the Gαi2 and Gαo proteins are impacted by these natural and pathological VNO changes. Our immunohistochemical studies showed that the Gαi2 was downregulated during aging process in mice, at 24 months compared to 3 and 10 months ($p<0.001$, Kruskal-Wallis test). Inversely, the Gαo was increased during aging, at 10 and 24 months ($p<0.001$, GLMM). Gαi2 downregulation in aging mice was also confirmed by in situ hybridization analysis ($p<0.001$, MOLR). Moreover, old mice seemed to be less able to detect the 2-heptanone, an alarm chemical cue specifically detected by the V1Rs. In a murine model of VNO inflammation, the Gαi2 expression was not impacted, while the Gαo was increased in the inflamed VNOs ($p=0.0114$, GLMM). These findings suggest that the Gαi2 protein expression is negatively affected by degenerative and inflammatory changes, while the Gαo protein is upregulated in affected VNOs, which could be due to compensatory mechanisms. In addition to the murine model, we have investigated the presence of Gαi2 protein also in farm animals. In pigs, the spontaneous VNO inflammation was strongly associated with the loss of Gαi2+ neurons in the vomeronasal sensory epithelium (VNSE), expressed as the number of Gαi2+ cell in 1 mm2 ($p<0.0001$, one-way ANOVA). Interestingly, the degree of the Gαi2+ neuron loss was also associated with inflammation intensity and with the reduction in VNSE thickness. In sheep, our preliminary findings showed that the number of Gαi2+ neurons was strongly reduced in inflamed VNOs (50.2 cells/mm2) compared to the healthy VNOs (98.3 cells/mm2), even if the difference was not statistically significant ($p>0.05$, GzLMM). Our findings showed that the G-proteins regulation is strongly impacted by natural and pathological changes of the VNO, although with a different pattern. Both Gαi2 and Gαo proteins play a crucial role in VNO functionality, thus their misregulation can negatively influence chemical cues detection in affected animals, inducing important changes in mammals chemical communication, behavior and ecology.

# Identification of olfactory neural substrates that promote aggressive behavior in male mice

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**Code:** 9
**Type of abstract:** TALK
**Keywords:** vomeronasal, aggression, V2R, behavior, urine, G protein

Mice emit pheromonal cues that trigger aggression in conspecific males, primarily detected through sensory neurons in the vomeronasal organ (VNO) of the accessory olfactory system. Several molecules have been identified as male-specific aggression-promoting chemosignals, but the sensory receptors responsible their detection remain largely unknown. Here, we identify a single G protein-coupled receptor of the V2R gene family as a specific pheromone receptor. By adopting a herpes virus-assisted receptor overexpression approach in VNO sensory neurons, we find that this receptor is selectively responsive to male mouse urine. To further elucidate the role of the receptor in intact vomeronasal epithelium and under in vivo conditions, we use a tamoxifen-inducible Cre recombinase system to generate conditional gene-targeted mice in which vomeronasal sensory neurons expressing this receptor can be selectively visualized or ablated. We show that receptor-expressing neurons respond to urinary molecules. Furthermore, time-dependent depletion of receptor-expressing neurons in male mice leads to reduced intermale aggression, establishing a key role for this receptor in driving male territorial aggression. By integrating genetic tools with artificial intelligence-based protein structure prediction and ligand-receptor interaction modeling, we uncover molecular determinants governing vomeronasal receptor ligand binding and activation. Our findings identify a member of the V2R as a pheromone receptor that detects specific urine chemosignals and drives aggressive behavior in male mice.

# The Role of PNNs in CA2 Neuronal Dynamics During Social Odor Processing

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**Code:** 10
**Type of abstract:** TALK

Social recognition in rodents is a primarily chemosensory process centered on the hippocampal CA2, a region where perineuronal nets (PNNs) are thought to stabilize social memory circuits. In this study, we investigated how PNN degradation impacts the electrophysiological representation of social odorants within the CA2.
We performed in vivo extracellular recordings in awake, head-fixed female C57BL/6 mice using high-density multielectrode probes. To manipulate the extracellular matrix, mice received bilateral intra-hippocampal injections of either Chondroitinase-ABC (to enzymatically degrade PNNs) or Penicillinase (control). During recordings, animals were challenged with a diverse battery of social volatile cues, including urine from intact and castrated males, conspecific females, and self-urine. Our preliminary results demonstrate that PNN degradation significantly alters the neural dynamics triggered by social scents. These findings suggest that PNN integrity is a fundamental requirement for the stable representation of chemical cues, and that their disruption impairs the circuit's ability to accurately decode social identity through smell.

# Characterization of Perineuronal Nets Across the Social Brain Network in Naturally Cycling Female Mice

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**Code:** 11
**Type of abstract:** TALK
**Keywords:** Socio-sexual behavior, synaptic plasticity, progesterone receptor and ventromedial hypothalamus

Female sexual behavior is tightly coupled to reproductive capacity through cyclic fluctuations in ovarian sex hormones. Estrogen and progesterone act on defined neural circuits via their cognate receptors, driving multiple neuromodulatory effects, including plastic changes across brain regions that enable the cyclic regulation of sexual behavior. Of particular interest are the regions comprising the social brain network (SBN), an interconnected set of areas enriched in neurons expressing sex hormone receptors and critical for the regulation of diverse social behaviors, including mating. Here, we characterize fluctuations in the expression of perineuronal nets (PNNs), specialized extracellular matrix structures that limit the formation of new synapses while stabilizing existing connections. Notably, PNN expression has been reported to vary across the estrous cycle in regions such as the hippocampus and cortex.

In this study, we focused on PNN density surrounding progesterone receptor-expressing neurons (PR+) throughout the SBN in naturally cycling female PR-Cre/EYFP mice. By comparing PNN intensity around PR+ cells in receptive versus non-receptive females, we aimed to determine whether cyclical hormonal changes are associated with alterations in PNN expression.

Our results show no significant changes in PNN density in the posterodorsal medial amygdala, bed nucleus of the stria terminalis, or ventral premammillary nucleus. In contrast, PNN density was significantly increased around PR+ neurons during the receptive phase in the anterior ventromedial hypothalamus (aVMH), a recently characterized region involved in sexual rejection in mice that undergoes extensive synaptic remodeling across the estrous cycle. These findings provide new avenues for investigating the neural mechanisms underlying synaptic plasticity in a brain area that regulates the cyclical expression of sexual rejection in female mice.

# Distinct afferent signatures onto anterior and posterior VMHvl subdomains and their progesterone receptor-expressing neurons

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**Code:** 12
**Type of abstract:** TALK
**Keywords:** Retrograde Tracing, Rabies Virus, Whole-Brain Mapping, Sexual Behavior

Female sexual behavior is tightly coupled to reproductive state and requires the coordinated regulation of sexual receptivity and rejection across the estrous cycle. In mice, these opposing behaviors are controlled by distinct anteroposterior subdomains of the ventrolateral ventromedial hypothalamus (VMHvl): the posterior VMHvl (pVMHvl) promotes sexual receptivity, whereas the anterior VMHvl (aVMHvl) drives sexual rejection. Although progesterone receptor–expressing ($PR^+$) neurons in both subregions are essential for these behaviors, the circuit mechanisms underlying this anteroposterior functional segregation remain poorly understood. Here, we investigated whether anterior and posterior VMHvl subdomains receive distinct afferent inputs that could support their divergent roles.

Using classic retrograde tracing combined with whole-brain, machine learning–assisted quantification, we mapped the afferent connectivity of the anterior and posterior VMHvl. We found that while the two subdomains share a majority of their inputs, they also receive distinct and biased projections. The aVMHvl preferentially receives inputs from the anterior hypothalamic nucleus, paraventricular and peripeduncular thalamic nuclei, and the parabrachial nucleus, whereas the posterior VMHvl is preferentially innervated by the preoptic area, ventral premammillary nucleus, anteroventral periventricular nucleus, and lateral septum. To determine whether these afferents target behaviorally relevant neurons, we performed monosynaptic rabies tracing from $PR^+$ VMHvl neurons, which corroborated these domain-specific input signatures.

Together, our results reveal distinct afferent architectures onto anterior and posterior $PR^+$ VMHvl neurons, providing a circuit-level framework for understanding how hormonal state flexibly biases female sexual behavior toward receptivity or rejection.

# PROLACTIN PARTICIPATES IN THE CHEMOSIGNAL PROCESSING AND MODULATES SOCIAL BEHAVIOR

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**Code:** 13
**Type of abstract:** TALK
**Keywords:** prolactin, prolactin receptor, social chemosignals, female behavior, olfactory bulb

Processing of pheromones by the accessory and principal olfactory systems profoundly influences neuroendocrine responses that trigger social interactions1. Prolactin hormone (PRL) receptors (PRLR) are extensively expressed within both olfactory systems2,3,4,5, and evidence show that, in females, knocking out PRLR in olfactory epitheliums impairs the mate preference, suggesting an important role of PRL on olfaction. To better understand the role of PRL on the processing of social chemosignals we performed two approaches using female mice as a model. The first one aimed to reveal the expression of PRLR within the olfactory bulb (OB) during sexual maturation (puberty onset -PO-, sexual maturity -SM- and adulthood -A-), as well as the behavioral response of females exposed to male stimuli after PRL administration, the OB circuitry activation, and the intracellular pathway involved. In the second one, PRL levels were modulated during critical periods related to maturation (juvenile and prepubertal period), by using PRL and dopamine receptor agonist and antagonist chronic treatments (tx), to determine its participation in short- and long-term olfactory function. In the first approach, results indicate that PRLR expression within MOB remains constant during all maturational stages, but in AOB this expression decreases in A. Behaviorally, females that received PRL augmented the active exploration of the male stimuli, along with an increased activation of the main OB (MOB) mitral cells (MC). Accessory OB (AOB)-MC activation showed by control females was impaired after PRL. Centrally, medial amygdala (MeA), as the first central relay coming from the AOB, showed an augmented response to the male stimuli after PRL. Interestingly, ERK pathway was enhanced after odor exposure only in MOB4. For the second approach, tx with PRL and cabergoline during a juvenile period delayed the puberty onset and altered the OB cell response (measured by cFos immunoreactivity -ir-) after exposure to male stimuli. Activation of granular cells (GC) within the AOB was impaired and contrary, the MOB-MC cFos-ir was increased with both tx, suggesting that PRL participates in the maturation of the AOB-MOB circuits and promotes participation of the MOB5. Finally, we evaluated the discrimination and preference behavior or females at SM and in A after receiving tx with sulpiride and cabergoline during a prepubertal period. All females at SM discriminated female vs male odors regardless the tx, however, at A, only females that received sulpiride and cabergoline tx maintained this discrimination capacity. Olfactory preference for female, gonadectomized (GDX) males and intact males stimuli among all groups was similar during SM and A; when compared between stages, differences were evident in A only in the treated groups, where cabergoline treated females increased the preference for females vs GDX males and sulpiride group preferred intact vs GDX males. These results suggest that the juvenile and prepubertal periods might be critical for PRL actions to organize the OB circuits that support specific innate social behaviors. Overall, our results confirm that PRL participates in the processing of chemosignals and behavioral response, probably by switching the classical vomeronasal response to pheromones and activating the main olfactory system.

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# Olfactory crosstalk between acetylation and phosphorylation in Alzheimer´s disease

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**Code:** 14
**Type of abstract:** TALK
**Keywords:** Alzheimer’s disease, proteomics, phosphoproteomics, acetylomics, PTM crosstalk

Post-translational modifications (PTMs) are essential for the correct dynamism of biological processes by regulating protein function and activity, folding, stability, and signaling. Multiple PTMs can occur on the same protein, and their interplay, known as PTM crosstalk, adds complexity to protein regulation (1). Advances in mass spectrometry enable high-throughput analysis of PTMs, improving our understanding of their roles and interactions (2). In Alzheimer's disease (AD), PTMs research has mainly focused on the well-characterized neuropathological Tau protein, showing their crucial role in its regulation, structure, and aggregation (3–6). However, comprehensive PTM-omics profiling and crosstalk analysis to better understand proteome deregulation in AD remains largely unexplored. In the present work, we mapped and integrated the proteome, phosphoproteome, and acetylome of AD affected olfactory bulbs (OB) in order to characterize the PTM landscape and key protein regulation across AD progression.

Briefly, protein extracts were obtained from 50 post-mortem OB derived from non-demented (n=6F/4M), AD-Braak stage I (n=4F/6M), Braak II (n=5F/5M), Braak III (n=5F/5M) and Braak IV (n=5F/5M) subjects. After protein digestion, enrichment in phosphorylated and acetylated peptides were performed and high-resolution LC–MS/MS data were acquired from Orbitrap Exploris 480. Data analysis was performed using Spectronaut/SpectroMine, and Perseus software. Metascape and Ingenuity Pathway Analysis softwares were considered for biological interpretation.

We observed a progressive proteomic impairment across Braak stages, primarily affecting mitochondrial metabolism, neuronal signaling, synaptic plasticity, and neuroinflammation. Moreover, we identified more than 1500 differentially phosphorylated residues in each Braak stage, from which 334 were commonly phosphorylated across the disease. Regarding OB acetylome, acetylation changes progressively increased with disease progression, showing a global deacetylation in Braak IV. To identify key PTM crosstalk in AD, we focused on significant negative correlations between differentially acetylated and phosphorylated sites within a protein, and positive PTM correlations at unchanged protein levels in each Braak stage. Most of the selected proteins were known interactors of Tau, amyloid precursor protein (APP), or previously detected in Aβ plaques. Among their biological function, several candidates played essential roles in ion exchange channels, such as ANK2, SCN2B, SLC1A3, or ATP1A1, suggesting their implication in synaptic activity, or were involved in microtubule assembly and dynamics (KIF21B, MAP1A, HDGFL3, NAV3). Interestingly, the mitochondrial creatine kinase CKMT1A, implicated in energy transduction in high-demand tissues such as the brain, presented hypo-phosphorylation at serine 147 and its activating residue tyrosine 153 at Braak III and IV stages, suggesting its implication in mitochondrial metabolism dysfunction. Moreover, phosphorylation at these residues positively correlated with acetylation at lysines 148 and 337, indicating a possible crosstalk between them and highlighting these sites as promising targets for further investigation of their interplay and therapeutic potential.

Overall, this comprehensive multiomics profiling elucidates the contribution of PTMs to disease onset and progression and enhances our understanding of the PTM regulation of potential therapeutic targets in AD.

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# The Artificial Sense of Smell: Technologies and Applications

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**Code:** 15
**Type of abstract:** SPECIAL TALK
**Keywords:** artificial olfactory system, sensors, data processing

The sense of smell plays a fundamental role in human perception, enabling the detection and interpretation of complex chemical environments. Inspired by biological olfaction, artificial olfactory systems—commonly known as electronic noses (e-noses)—have emerged as powerful tools for the detection, identification, and monitoring of volatile compounds. This talk presents an overview of the technological foundations of artificial olfaction, focusing on the main functional components of an electronic olfactory system and its most relevant applications.

An artificial olfactory system typically consists of four main subsystems: sampling, sensing, electronic instrumentation, and data processing. The sampling method represents the interface between the environment and the sensing unit, determining the reliability and reproducibility of measurements. Sampling strategies may include static headspace sampling, dynamic flow systems, preconcentration techniques, or microfluidic approaches designed to control humidity, temperature, and analyte transport. Proper sampling is essential to ensure representative acquisition of volatile organic compounds (VOCs) while minimizing contamination and drift.

The sensor array forms the core of the artificial nose. Instead of relying on highly selective sensors, e-noses employ partially selective chemical sensors whose collective response generates characteristic patterns or “olfactory fingerprints.” Common sensor technologies include metal oxide semiconductor (MOS) sensors, conducting polymer sensors, quartz crystal microbalance (QCM) devices, surface acoustic wave (SAW) sensors, and electrochemical sensors. Each technology exhibits distinct sensitivities, response times, and operating conditions, allowing complementary detection of complex gas mixtures.

The electronic instrumentation subsystem conditions and converts sensor signals into usable digital information. This stage includes sensor excitation circuits, signal amplification, filtering, analog-to-digital conversion, and temperature or humidity compensation. Advances in embedded electronics and low-power microcontrollers have enabled portable and real-time olfactory platforms suitable for field deployment and Internet-of-Things (IoT) integration.

Finally, data processing and pattern recognition transform multidimensional sensor responses into meaningful chemical information. Techniques such as principal component analysis (PCA), linear discriminant analysis (LDA), artificial neural networks (ANNs), support vector machines (SVMs), and deep learning algorithms are widely used for classification, regression, and anomaly detection. Machine learning plays a crucial role in compensating for sensor drift, improving selectivity, and enabling adaptive learning in dynamic environments.

Artificial olfactory systems are currently applied across diverse domains. In the food and beverage industry, they support quality control, freshness assessment, and adulteration detection. In environmental monitoring, e-noses enable detection of air pollutants, hazardous gases, and odor nuisance evaluation. Medical and biomedical applications include non-invasive disease diagnosis through breath analysis. Additional applications arise in industrial process control, agriculture, security and defense, smart packaging, and robotics, where olfactory sensing enhances autonomous decision-making.

# Modeling Human Neurogenesis through 3D Bioprinted hiPSC Constructs

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**Code:** 16
**Type of abstract:** TALK
**Keywords:** Bioprinting; 3D; Cortex; hiPSC; Neurodevelopment

Through the controlled layer-by-layer deposition of biomaterials, 3D bioprinting provides a precise and tunable platform for engineering cellular microenvironments that support critical cell-cell and cell-matrix interactions. This technology enables the fabrication of complex 3D constructs, mimicking native tissue characteristics with high reproducibility and scalability[1]. In the present study, we describe a novel approach to create neural tissue-like constructs by bioprinting human iPSCs for in situ neural differentiation. Constructs were generated through extrusion printing a bioink composed of Geltrex™: 8% Gelatin methacrylate (GelMA) (1:1), followed by ultraviolet (UV) light cross-linking. Post-printing, the 3D constructs were incubated in Gibco Essential 8 (E8) Medium for stemness maintenance or subjected to neural induction through dual SMAD inhibition and further neural expansion and neuronal maturation. Post-printing live/dead assays demonstrated high cellular viability within the constructs. In cells maintained in E8 medium, the sustained expression of pluripotency markers, including OCT4 and NANOG, was validated via RT-qPCR and immunostaining. Throughout the neural induction phase, significant morphological transitions were observed: cells transitioned to a bipolar morphology characteristic of neural progenitor cells (NPCs), and time-lapse imaging confirmed active cellular migration within the 3D scaffold. The reorganization of the cytoarchitecture was further evidenced by the development of neural rosettes. To evaluate differentiation progress, we are performing quantitative morphological and phenotypic assessments at 30 and 60 days post-printing using RT-qPCR and immunophenotyping. By day 120, the constructs displayed clear cortical layering markers, including CTIP2 (deep layer) and SATB2 (upper layer) neurons, S100B+/GFAP+ glial populations are also present in this age. Long-term cultures were maintained for up to 170 days, with multielectrode array (MEA) recordings detecting electrophysiological activity as early as day 60. This approach offers a reproducible and adaptable platform for modeling human development, allowing for the exploration of diverse geometries and scales that are typically restricted in conventional organoid models. By leveraging hiPSCs, this bioprinting strategy enables the generation of multiple tissue types from a single batch, significantly reducing experimental variability. Consequently, it provides a scalable framework for investigating tissue morphogenesis and organogenesis, with robust applications in drug discovery and regenerative medicine. Financial support: São Paulo State Research Foundation (FAPESP; 2018/12605-8 to M.A.P; 2023/14105-0 and 2025/25725-5 to N.H.O.) and National Council for Scientific and Technological Development (CNPq; 406258/2022-8 to M.A.P., INCT Model 3d)

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# Nasal Swab-Derived Olfactory Cultures: A Minimally Invasive Model for Disease Study

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**Code:** 17
**Type of abstract:** TALK

The Olfactory Epithelium (OE) serves as a unique window into the central nervous system, containing neural progenitor cells and olfactory sensory neurons that are directly accessible. Traditional methods for obtaining these cells often involve invasive biopsies; however, nasal swabbing has emerged as a safer, minimally invasive alternative. This study presents preliminary data on the successful isolation and expansion of primary cell cultures derived from human nasal swabs, evaluating their potential as a robust platform for disease modeling. Using a standardized non-invasive protocol, samples were collected from healthy donors and processed to isolate primary cells, which were subsequently cultured in specialized media to promote the expansion of both mesenchymal-like stromal cells and olfactory neural progenitors. Our preliminary results demonstrate that viable cell populations can be consistently retrieved and expanded from a single nasal swab, showing high proliferative capacity and a phenotypic diversity that includes markers consistent with olfactory lineage cells and neural progenitors. These initial findings suggest that nasal swab-derived cultures effectively capture the cellular components of the OE, offering a promising cellular representative of the olfactory system. This points cultivation of OE cells from nasal swabs as a promising tool for translational research, offering significant potential for modeling neurodevelopmental disorders and rare diseases that require patient-specific samples to advance personalized medicine.

# Validation of a new olfactory test for neurodegenerative patients

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**Code:** 18
**Type of abstract:** TALK
**Keywords:** Human olfaction, olfactory test, Parkinson’s disease, Alzheimer’s disease

Olfaction in humans remains still an elusive sense. Nevertheless, olfactory impairments in patients as well as changes in their olfactory capabilities have been considered hallmarks of different neurodegenerative disorders. On this basis, olfactory tests appear as tools to evaluate human olfactory perception, both for diagnostic and research purposes. Olfactory tests for humans are diverse, and some of them well-known amongst the scientific community, as the University of Pennsylvania Smell Identification Test (UPSIT). A common element for most of the human olfactory tests is the number of trials (sometimes with more than 40), which makes them very specific and sensible, but notably increases the time to be completed. Then, although they are very useful for research purposes, providing many and varied data, their application in a standard medical consultation –with very limited time- is completely unaffordable. Another limitation of the available human olfactory tests to date is the randomization of their odorants, whose selection is usually conditioned to the own researcher’s criterion, expertise or culture, but lacking any scientific base. Then, this odorant selection is frequently biased. These limitations finally lead to adaptations of olfactory tests to different countries, including the development of longer/shorter versions, which finally increases data variability.

We have developed a new olfactory test with just 10 odorants, based in the odor classification according to Castro et al. (2013). To the best of our knowledge, our work is unique by employing an accurate scientific classification of odorants in 10 categories, based on hierarchical descriptors. The short extension of this test (just 10 trials) as well as the methodology for its administration allows its use in a common consultation of Otorhinolaryngology, Neurology or general Medicine. The test has been in the process of validation/refinement after being passed in a cohort of more than 250 healthy volunteers. In this work, our test has also been employed in two independent cohorts of patients of both Alzheimer’s and Parkinson’s neurodegenerative diseases, revealing clear differences in their patters of olfaction when compared to healthy volunteers.

Altogether, our data support this new olfactory test as a strong proof for evaluating olfaction in humans, also being useful for medical testing and diagnoses.

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# The human olfactory bulb in Huntington’s disease: sex-specific differences in the volume and gliosis

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**Code:** 19
**Type of abstract:** TALK
**Keywords:** Astroglia, microglia, olfaction, stereology, Vonsattel grades

Huntington’s disease (HD) is a rare inherited neurodegenerative disorder characterized by motor disturbances, including choreiform movements, primarily associated with striatal pathology [1]. Despite this, aggregates of mutant huntingtin have been identified in multiple brain regions beyond the striatum, such as the olfactory bulb (OB) [2], a layered structure that contains the anterior olfactory nucleus (AON), a highly connected hub critical for olfactory processing. This may be related to early non-motor manifestations, including hyposmia, an early alteration observed in several neurodegenerative diseases [3]. However, OB involvement in HD and the possible influence of sex-related factors have not yet been fully characterized. This study aims to stereologically analyze volumetric and glial (astroglia and microglia) alterations in the OB in HD, considering sex-related differences.

A total of 30 post-mortem fixed OB samples from non-disease and disease patients were analyzed. Histological methods (Nissl staining), immunohistochemical techniques (glial markers, GFAP for astroglia and Iba1 for microglia), and stereological approaches (Cavalieri’s estimator and the area fractionator method) were applied to assess volume and gliosis across the entire OB and its different layers. The results showed similar volumes between women and men. However, a negative correlation between OB volume and Vonsattel grades in the AON was observed only in women. A trend toward astrogliosis was detected exclusively in women, whereas significant microgliosis was observed in the overall disease population, remaining significant only in men when analyzed by sex. These findings suggest that the OB, particularly the AON, is affected in HD and reveal sex-specific patterns in volumetric and glial pathology, highlighting the importance of considering biological sex as a critical variable in future research.

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# Olfactory proteomics reveals the capacity of the HDAC1 inhibitor Pyroxamide to halt the α-synuclein preformed fibrils-induced damage in human nasal epithelial cells.

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**Code:** 20
**Type of abstract:** TALK
**Keywords:** alpha-synuclein, drug repurposing, olfactory dysfunction, Parkinson's disease, proteomics, pyroxamide

Parkinson's disease (PD) is the second most common neurodegenerative disorder mainly characterized by the degeneration of dopaminergic neurons originating in the substantia nigra (SN) pars compacta and projecting to other brain regions, giving rise to motor and non-motor symptoms [1-5]. Despite significant progress in understanding the molecular and cellular disruptions associated with PD, there remains an unmet clinical need for effective therapies [6,7]. In this study, proteomic analysis of the olfactory tract (OT) in controls with no known neurological history (n=17) and PD subjects (n=21) revealed Lewy body disease (LBD) stage-dependent proteostatic impairment, accompanied by progressive modulation of the alpha-synuclein (α-syn) functional interactome. Differential OT omic profiles (OMS) were used in a computational drug repurposing approach, reveling the HDAC1 inhibitor pyroxamide as one of the top drug candidates with in silico potential to restore altered OMS [8-11]. To explore the potential therapeutic effects of pyroxamide, in vitro assays were performed using α-syn preformed fibrils (PFFs). Pyroxamide treatment produces a protective effect against α-syn PFFs-induced toxicity in olfactory epithelial cell line. These findings confirm the suitability of omics profiles in drug repurposing workflows against PD, offering valuable insights into the potential of HDAC1 inhibitors in the therapeutic pipeline of PD.

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# Sobre las Bases Genéticas de la Sensibilidad Química Múltiple

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**Code:** 21

**Type of abstract:** SPECIAL TALK

**Keywords:** SLC gene superfamily; chemical intolerance; multiple chemical sensitivity; olfactory hyperactivation; sensory hyperexcitability; whole-exome sequencing

(On the Genetic Basis of the Multiple chemical sensitivity)

Multiple chemical sensitivity (MCS) is a disease of unknown etiology with multiple symptoms. Triggered by exposure to environmental chemicals, it results in multiorgan effects. Studies on MCS use different approaches, ranging from searches for environmental triggers to susceptibility genes. Genetic research undergoes two different directions, either a predictive approach looking for genetic variants in genes related to some previously described symptoms in patients or a general search for genetic variants especially present in the patients that requires a big sample of patients. Until now, the predictive approach deals with genes for chemical detoxification, oxidative stress, inflammation, and neurodegeneration, as well as immune function and mast cell activation, with uneven results. The olfactory hyperexcitability symptom, reported in more than 90 % of the MCS cases, was not considered yet because of the lack of known genes associated with this symptom. However, it has recently been linked to a member of the SLC (Solute Carrier) gene superfamily. To explore its role in MCS disease, a whole-exome analysis was performed in a small number of subjects. Low-frequency genetic variants were analyzed for each individual, and their homozygous or heterozygous presence was determined in four groups of genes related either to the SLC superfamily members or to previous studies in MCS. We found homozygous rare variants in affected individuals only for the SLC gene superfamily, where each patient had at least one. Variants in heterozygosis and certain SNPs also point to SLC genes related to neurotransmitter synthesis, release, and clearance, as well as to the level of cellular excitability, as potentially underlying the differences.

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# Cajal: Curiosidad, Ciencia y Legado

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**Code:** 22
**Type of abstract:** SPECIAL TALK

Para que se pueda asimilar y aprender se necesita emoción, y para que se puedan hacer descubrimientos se necesita curiosidad. Cajal fue una persona extremadamente curiosa y ponía una desmesurada pasión en todo trabajo que acometía, lo que se tradujo en una obra científica que tuvo una amplia difusión y un gran reconocimiento internacional. Desgranar su amplia contribución científica y su repercusión es tarea que requiere bastante tiempo, aparte de ser mayormente conocida por este foro al cual me dirijo. Por lo tanto, en la presente comunicación me voy a limitar a enumerar sus descubrimientos mas rompedores en el estado de la neurociencia en aquella época y a comentar algunas anécdotas poco conocidas que acontecieron como resultado del reconocimiento a su trabajo.

# Neural Stem Cell Quiescence in the Olfactory System

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**Code:** 23
**Type of abstract:** PLENARY LECTURE
**Keywords:** Neurogenesis, V-SVZ, extracellular matrix, mechanotransduction, YAP/TAZ

New neurons that sustain the highly plastic olfactory circuitry are generated in the subependymal zone (SEZ) of the adult mammalian brain. Neural stem cells (NSCs) within this niche are exposed to a broad array of regulatory cues that support lifelong neurogenesis while maintaining the stem cell reservoir. Adult NSCs originate from radial glial cells, the principal embryonic progenitors in the vertebrate brain, and retain key features of this lineage, including elements of their transcriptional program, a bipolar elongated morphology with apico-basal polarity enabling specialized interactions with neighboring cells, and expression of astrocytic markers. Unlike their fetal counterparts, however, most adult NSCs remain quiescent under physiological conditions. Transitions between quiescence and activation likely entail profound shifts in cellular physiology governed by tightly coordinated intrinsic and extrinsic mechanisms. We have found that entry into quiescence is accompanied by the deposition of specific extracellular matrix (ECM) components. Moreover, adhesion to ECM produced in response to pro-quiescent signals is sufficient to induce a quiescent-like state in proliferating NSCs. This transition requires RhoA-associated kinase (ROCK) activity and yes-associated protein (YAP)–dependent transcription. Deletion of YAP/TAZ in NSCs results in impaired ECM deposition and loss of quiescence in vivo, indicating that these factors orchestrate both the physical properties of the niche and a quiescence-associated transcriptional program in response to mechanical cues.

# A Pax6 isoform switch underlies the transition from neural progenitor to mature neuron identity

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**Code:** 24
**Type of abstract:** TALK
**Keywords:** Pax6, alternative splicing, neurogenesis, neuronal differentiation

The transcription factor Pax6 presents a fundamental paradox in the adult brain. In the subventricular zone (SVZ), Pax6 drives neural progenitor proliferation and is sufficient to reprogram mature astrocytes into progenitor-like cells. Yet in postmitotic neurons of the olfactory bulb and olfactory cortex, Pax6 is robustly expressed without disrupting differentiated identity. How the same transcription factor produces opposing cellular outcomes at different stages of neuronal differentiation has remained unresolved.

We hypothesized that this functional divergence is encoded at the isoform level. The three major Pax6 isoforms differ in their DNA-binding domains and therefore in their transcriptional targets, making them strong candidates for mediating stage-specific functions. To test this, we generated CRISPR knock-in mouse lines in which each isoform is individually tagged with a short epitope, enabling single-isoform resolution in vivo for the first time. We find that SVZ progenitors predominantly express the canonical Pax6 isoform, while differentiation is accompanied by a progressive, cell-autonomous shift toward Pax6(5a) in mature neurons. This transition is temporally coupled to defined maturation stages along the SVZ-to-olfactory bulb axis. Importantly, the same isoform switch is observed in other Pax6-expressing structures, including the epithalamus and olfactory cortex, suggesting that it represents a general feature of neuronal differentiation rather than a circuit-specific phenomenon.

These findings reveal a previously uncharacterized dynamic in Pax6 isoform composition during neuronal maturation, and raise the possibility that the canonical isoform and Pax6(5a) support distinct stages of neuronal identity. Future functional studies will be needed to determine how each isoform contributes to progenitor maintenance and mature neuron identity, with potential implications for Pax6-linked neurodevelopmental disorders and neural regeneration.

# Rostral neurogenesis in a murine model of Autism Spectrum Disorder by administration of valproic acid

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**Code:** 25
**Type of abstract:** TALK
**Keywords:** autism, adult neurogenesis, olfactory bulb, rostral migratory stream, subventricular zone, VPA model.

Autism Spectrum Disorder (ASD) is a neurodevelopmental condition with a seemingly increasing incidence in recent years. Although its etiology remains unclear, it is widely accepted to result from complex gene-environment interactions. Animal models are essential for its study, the valproic acid (VPA)-induced murine model being the most widely used due to its replication of ASD-like behavior. In this model, VPA has been shown to reduce cell proliferation in the hippocampus, a key region for adult neurogenesis. However, its effect on the subventricular zone (SVZ), another neurogenic region, and the subsequent integration of neuroblasts into the olfactory bulb (OB) remains underexplored. Moreover, previous findings from our research group show that the VPA model experiences a significant reduction of nNOS interneurons in the glomerular layer (GL) of the OB, which made us wonder whether VPA could impair the neurogenic process of this region.

To address this question, we performed a complete analysis of the whole neurogenic process, starting from the proliferation of cells in the SVZ to their migration and integration into the GL of the OB as differentiated nNOS cells. We started by quantifying the density and percentage of Ki67 positive cells in the SVZ and rostral migratory stream. Then, the quantification of doublecortin positive neuroblasts in the GL were analyzed to explore the early migration and differentiation. Furthermore, we tracked terminal differentiation by quantifying BrdU and nNOS positive co-expression.

Finally, to determine whether observed cell reduction could be also due to early programmed cell death, we performed a TUNEL/nNOS+ double-labeling. Our analysis of the initial stages of neurogenesis in the SVZ and rostral migratory stream showed no significant differences between the control and VPA groups, suggesting that VPA does not influence the beginning of the neurogenic process in the OB. However, a significant decrease in the number of new nNOS-positive cells that reached and integrated into the GL was observed in the VPA group when compared to controls.

Additionally, although without full statistical significance, a tendency to a higher rate of apoptosis in nNOS-positive cells of the VPA group was also detected.

Our findings suggest that while the starting point of the neurogenic SVZ-OB pathway remains unaltered by VPA, the later stages of migration and terminal differentiation of nNOS interneurons in the OB seem to be disrupted by this substance. These results may be on the basis of the sensorial impairments -olfaction, in this case- that characterize ASD.

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# Spatial Organization and Morphometry characterization of NG2 glia in the olfactory bulb

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**Code:** 26
**Type of abstract:** TALK
**Keywords:** NG2 glia; olfactory bulb; morphology

NG2-glia represent a widespread and highly dynamic population of glial cells in the central nervous system traditionally classified as oligodendrocyte precursor cells (OPCs). Beyond their role as progenitors, accumulating evidence indicates that these cells exhibit remarkable morphological plasticity, establish extensive contacts with neurons and glial cells, and participate in local circuit dynamics. Recent work has revealed that NG2 glia display layer-specific morphologies in the cerebral cortex, suggesting that local microenvironments may shape their structural organization. Whether similar principles govern NG2 cell organization in other brain regions remains largely unexplored.

The olfactory bulb provides an ideal system to address this question. This structure exhibits a highly defined laminar architecture composed of distinct neuronal and glial populations and is characterized by pronounced structural plasticity and continuous adult neurogenesis.

In this study, we analyze the morphology and spatial organization of NG2-glia across the different layers of the olfactory bulb, using confocal microscopy and immunolabeling for markers including Olig2, PDGFRα and Sox10. In parallel, we developed a computational pipeline based on Napari and Python to perform automated segmentation, morphometric quantification and spatial mapping of NG2 cells across regions, allowing us to explore potential layer-dependent organizational patterns.

Our preliminary results reveal layer-dependent differences in both the morphology and density of NG2 glia. These findings suggest that NG2 cells undergo structural adaptations influenced by the local cellular microenvironment and the functional architecture of olfactory circuits. Together, these observations provide new insights into the spatial organization of NG2 glia and highlight the potential role of local circuit architecture in shaping glial morphology within the olfactory bulb. This work was supported by the grant PID2022-136882NB-I00, funded by MICIU/AEI/ 10.13039/501100011033.

# Following the Neurogenic Route: NG2-Glia in the OB–RMS–SVZ Axis

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**Code:** 27
**Type of abstract:** TALK
**Keywords:** NG2 glia, Rostral Migratory Stream (RMS), Synaptic plasticity, Neuroblast migration

NG2 glia are increasingly recognized as a distinct macroglial population in the central nervous system, extending beyond their classical role as oligodendrocyte precursor cells (OPCs). Recent studies have highlighted their potential capacity to modulate synaptic activity through direct neuronal inputs; however, their broader role within specialized neurogenic niches remains to be fully elucidated. One particularly intriguing yet understudied, feature of these cells is their strategic localization within the Rostral Migratory Stream (RMS). In this pathway, NG2 cells display a specialized morphology, and align closely with the GFAP+ astrocytic "glial tube" that guides neuroblast migration from the subventricular zone (SVZ) to the olfactory bulb (OB).

Given the olfactory bulb’s role as a major site of synaptic plasticity and neural integration, we investigated the potential contribution of NG2 glia to these processes. Using NG2-Tdt reporter mice, we performed a detailed immunohistochemical analysis of GFAP+ scaffolds and DCX+ neuroblasts across the OB-RMS-SVZ axis. To resolve interactions between NG2 processes and nascent circuits at the nanoscale, we employed ultra-resolution TauSTED-Xtend imaging of synaptic markers across the OB layers. In parallel, StarTrack multi-color lineage tracing was used to define the progeny of SVZ progenitors during embryonic development.

Our preeliminary findings reveals a complex spatial and functional integration of NG2 glia within this system, suggesting that these cells may contribute to the structural organization and synaptic assembly of the adult olfactory circuitry. This work was supported by the grant PID2022-136882NB-I00, funded by MICIU/AEI/ 10.13039/501100011033.

# Postnatal development of vasopressinergic innervation in the olfactory amygdala and related social olfactory behaviours in a mouse model of Rett syndrome.

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**Code:** 28
**Type of abstract:** TALK
**Keywords:** Nonapeptides; Social behaviour; Rett syndrome

Rett syndrome is a severe neurodevelopmental disorder primarily caused by mutations in the X-linked *MECP2* gene and characterized by cognitive and motor impairment. Mouse models of Rett syndrome recapitulate important aspects of the disease, including altered social behaviours and stereotyped and repetitive movements such as excessive grooming. These behaviours are modulated by the nonapeptidergic systems of vasopressin (AVP) and oxytocin (OT), which play a central role in the control of social behaviours, including olfactory recognition in mice. Previous studies in the B6.129.*Mecp2*-null mouse model for Rett syndrome showed a dysregulation of these neuropeptidergic systems and impairment of olfactory investigation. Here, we characterized the AVP-ergic and OT-ergic systems longitudinally in a Rett syndrome mouse model derived to a CD1 background. We analysed key developmental stages, including minipuberty during early postnatal life (P12), pubescent stage (P28), and young adulthood (P60), using double immunofluorescence labelling for AVP and OT and analysing brain regions critically involved in social behaviour and olfaction, including the medial extended amygdala and posterodorsal medial amygdala. In parallel, we assessed the olfactory impairment by testing investigation of urine chemosignals from different males by means of a habituation-dishabituation test. Our results provide novel information of the postnatal development of nonapeptidergic systems in the mouse model for Rett syndrome and their possible relationship with altered social behaviours. Funded by Conselleria de Educación, Cultura y Universidades from the Generalitat Valenciana (CIAICO/2023/027; CIAICO/2023/041) and the Spanish Ministry of Science, Innovation and Universities (PID2022-141733NB-I00; MCIU/ AEI /10.13039/501100011033/FEDER, UE).

# Synaptic communication between microglia and neurons across degenerative processes of a model of selective neuronal death, the PCD mutant mouse.

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**Code:** 29
**Type of abstract:** TALK
**Keywords:** Purkinje cells, Microglia, Synaptic plasticity, Neurodegeneration, PLX5622, Cerebellum, Olfatory Bulb,

Synaptic stability in the cerebellum depends on tightly regulated interactions between neurons and glial cells. In this regard, microglia plays a key role in synaptic pruning, formation and maturation, yet its functions during neurodegeneration remain incompletely understood. Here we investigated how microglial inhibition affects synaptic organization using the Purkinje Cell Degeneration (PCD) mutant mouse. The PCD model presents two distinct neurodegenerative scenarios within the same organism. In the cerebellum, the mutation causes rapid Purkinje cell degeneration, from postnatal day 18 (P18) to P40, providing an ideal system to study acute synaptic changes due to neurodegenerative processes. In contrast, degeneration in the olfactory bulb (OB) occurs later and progresses more slowly (P60–P110), resembling an standard age-related neurodegeneration. The cerebellar scenario constitutes the starting point of our study due to the speed with which results can be obtained. Future work will extend these analyses to OB, to determine how severity and temporal progression shape microglia-dependent synaptic remodeling. We analyzed synaptic organization at a pre-degenerative stage (P15) and peak degeneration (P25) using gene expression and protein detection of the excitatory postsynaptic marker PSD-95. qPCR showed that both the pcd mutation and PLX5622 reduce transcription of Psd-95. However, immunohistochemistry revealed a different pattern: in PCD mice, synaptic puncta density increased before neuronal death, suggesting early over-synaptogenesis or protein accumulation. Notably, at maximal degeneration, neuronal loss was not accompanied by decreased PSD-95 protein. High-resolution analyses combining Calbindin D28K, PSD-95, and Iba1 immunostaining quantified Purkinje neurons, synaptic puncta, and microglial distribution. At P15, PCD mice displayed increased synaptic puncta, consistent with an early compensatory response. Colocalization analyses revealed that pre-degenerative signals trigger an early microglial response, with microglia prioritizing protective interactions with neurons over synaptic maturation. At P25, microglial depletion produced context-dependent effects. In controls, PLX5622 impaired synapse establishment, confirming microglial roles in normal maturation. In PCD mice, this effect was absent; instead, treatment increased excitatory synaptic puncta density only under active pathology. Surviving microglia localized preferentially at synapses, suggesting a protective or compensatory function. Moreover, at P25, PLX5622 reduced microglial contacts with Purkinje somata, while residual microglia concentrated around persistent synaptic puncta, prioritizing synaptic preservation. Both microglial depletion and dendritic atrophy altered microglia–synapse colocalization along dendrites. Overall, these findings reveal an early compensatory synaptic phase preceding neuronal death and identify a microglia-dependent neuroprotective mechanism during degeneration. Microglia surviving pharmacological depletion adopt adaptive protective roles in pathological environments, reorganizing their interactions to preserve vulnerable synaptic circuits. Ongoing analyses in the OB will demonstrate whether this pattern of microglia distribution and behavior is maintained in a milder neurodegenerative scenario.

# Neuroprotective effects of VEGF-B in the mitral cells of PCD mouse

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**Code:** 30
**Type of abstract:** TALK
**Keywords:** Neurorepair, VEGF-B, olfactory bulb, mitral cell, PCD mouse

Neurodegenerative diseases represent a major challenge and require substantial economic investment in both social and healthcare systems. Our group has
extensive experience in the analysis of an animal model used to develop neuroprotective strategies, the PCD mouse, which suffers neuronal death affecting the olfactory bulb (OB). Such degeneration mimics some of the most prevalent features of human neurodegenerative processes, as it is late-onset and prolonged over time. Here, we aimed at demonstrating the neuroprotective properties of Vascular Endothelial Growth Factor Type B (VEGF-B). Specifically,
we investigated whether systemic administration of this neurotrophic factor could reduce neuronal death in the OB of PCD mice. To this end, two different
schedules of periodic intraperitoneal VEGF-B administration were performed: from postnatal day 40 (P40) to P90 or from P50 to P90, with injections every five
days. Animals underwent the new odour identification behavioural test, an ad hoc modified new object recognition test and were subsequently sacrificed for
immunofluorescence analysis of the OB. Preliminary results suggest that VEGF-B increases mitral cell survival in PCD mice, as evidenced by an increase in
neuronal number, soma size, and dendritic trunk diameter. These morphological changes may be reflected in improved olfactory capabilities. In addition, the
density of vascular endothelial growth factor receptor 1 in mitral cells shows an opposite distribution, with higher expression in untreated PCD mice. Overall,
these findings represent a further step toward the development of effective neuroprotective therapy using VEGF-B. Moreover, we are now developing new
systems of VEGF-B though its overexpression in transplanted bone marrow stem cells.

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# Fine-tuning of bone marrow-derived microglia cell cultures for VEGF-B overexpression under specific promoter

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**Code:** 31
**Type of abstract:** TALK
**Keywords:** BMDML, bone marrow, cell culture, microglia, olfactory bulb, VEGF-B

Microglia carries out key roles for maintaining the homeostasis and functionality of the central nervous system, exhibiting considerable plasticity in response to the physiological or pathological environment in which they are located. Apart from their neural origin, in adult organisms, microglia can be originated from hematopoietic precursors. Indeed, it is feasible to obtain these cells in vitro from cultures of adult hematopoietic cells, thereby generating the so-called bone marrow-derived microglia-like (BMDML) cells, by simulating a neural environment. Besides, in our research unit, evidence has been presented demonstrating that modulation of microglial activity by cell therapy can reduce various processes of neuronal death (including the olfactory system). These results foster the use of BMDML cells as therapeutic tools. In another parallel research line, the expression of the neurotrophic factor VEGF-B has demonstrated to be altered in a mouse model of selective death of mitral cells of the olfactory bulb (OB). When VEGF-B was intraperitoneally administered, it reduced mitral cell loss. These results have given rise to the consolidation of a new line of work focused on the expression of this neurotrophic factor in bone marrow-derived cells, in search of a synergic protective effect on the neurodegeneration of the OB. The initial phase of this research field is the optimization of BMDML production and their infection with lentiviruses for the overexpression of VEGF-B. To this end, a range of conditioned media have been tested to promote BMDML cell differentiation. The age of the animals used to establish cultures of brain cortex (for obtaining culture medium), the frequency of renewal of the culture medium, and the proportion of fetal bovine serum added to the cortex culture have been identified as key factors. The feasibility of genetic modification and overexpressing VEGF-B has been demonstrated without any apparent alteration in differentiation to BMDML. This optimized VEGF-B-overexpressing BMDML production sits the bases for transplants of hematopoietic cells modified for the overexpression of VEGF-B in vivo to study its potential neuroprotective effect on the OB.

# Characterization of splenic immune populations and bone marrow transplant-induced shifts in a model of olfactory bulb degeneration

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**Code:** 32
**Type of abstract:** TALK
**Keywords:** Spleen immune system, olfactory bulb degeneration

The communication between the immune and central nervous systems may be altered during neurodegeneration. The spleen, a major reservoir of immune cells, plays a key role in filtering blood and modulating systemic lymphocyte activation. In the Purkinje Cell Degeneration (PCD) murine model, which exhibits a dual loss of Purkinje and mitral cells, we have previously characterized an immune response during cerebellar degeneration. Building on this, we aim at investigating the peripheral immune shifts during the gradual degeneration of the olfactory bulb (OB) and exploring the potential modulatory effects of bone marrow transplantation (BMT), which has been shown to improve mitral cell survival. We performed a longitudinal flow cytometric analysis of splenic myeloid and lymphoid populations during OB degeneration, including a cohort subjected to BMT after irradiation.

Initial results indicate that spleen immune cells alterations during OB degeneration are not as numerous as those previously described during cerebellar degeneration. However, we have detected a specific early activation of splenic CD4+ cells at the onset of the neurodegenerative process. On the other hand, preliminary BMT data reveal a systemic shift characterized by a restricted expansion of myeloid compartment in PCD mice compared to WT, alongside a transition toward a more granulocytic profile.

Our findings indicate that PCD neurodegeneration impacts the peripheral immune system in a region-specific and severity-dependent manner. Preliminary data suggest BMT can subtly reshape the systemic immune landscape at the neurodegeneration onset. Understanding of these interactions could offer novel insights into regulating the immune response in these disorders.

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# Functional alterations in olfactory ability during kidney disease progression

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**Code:** 33
**Type of abstract:** TALK
**Keywords:** Olfactory alterations, behavioral olfactory tests, kidney disease, systemic inflammation.

Mexico has a high prevalence of kidney disease (KD). KD is characterized by functional and structural abnormalities of the kidneys1,2,5. KD patients report olfactory alterations that affect their quality of life and can lead to unbalanced feeding behavior, altering metabolism1,4. The onset and evolution of the pathophysiology of the olfactory alterations have been little addressed. In the present study, a KD model was generated in adult C57BL/6J female mice by oral administration of adenine (tx; 50 mg/kg, n=10)5. To assess olfactory alterations in this model, we employed four behavioral tests: the olfactory threshold test (OTT), the habituation/dishabituation test (HDT), the olfactory food preference test (OFPT), and the buried food test (BFT). These tests evaluated the mice's ability to detect (sensitivity-OTT and locate food-BFT using olfactory cues), differentiate odors (discrimination process-HDT), and their preference (olfactory motivation-OFPT)3,6. The tests were performed before the tx and 4, 6 and 8 weeks after adenine-tx. Before adenine-tx, all female mice showed intact olfactory abilities. Four weeks after adenine-tx, mice displayed decreased odor sensitivity-OTT, showing reduced sniffing time and decreased HDT performance over time, becoming more pronounced at 8 weeks after adenine-tx. Regarding BFT, in week 8 after adenine-tx, mice displayed a decrease in the latency for uncovering the buried food. Interestingly, 4 and 6 weeks after adenine-tx, KD mice showed an increase in time spent eating the buried food. In OFPT, neither group showed any olfactory food preference at 4, 6, and 8 weeks after adenine-tx. However, we observed that the percentage of the total exploration time to each food stimulus decreased in KD mice. Additionally, since KD generates a systemic inflammatory response2, and inflammation has been associated with morphological and functional deterioration of olfactory structures, circulating levels of IL-6 were determined by ELISA during the progression of the KD at 1, 2, 3, 4, and 8 weeks after adenine-tx. As expected, peripherical levels of IL-6 increased in adenine-treated mice from weeks 1 and 2 and decreases significantly by the 8th week, returning to basal levels. Our data indicates that adenine-tx disrupts olfactory function in mice altering the detection and discrimination processes. Also, our results showed that IL-6 levels rise as part of the proinflammatory response probably as a cause of the accumulation of crystals of adenine metabolite that could activate immune cells and promote cell death. Low levels of IL-6 levels do not mean absence of inflammation, and interestingly, when IL-6 decreases, acute inflammation diminishes, a moment coincident with the increase of profibrotic mediators increase. Further experiments are needed to unravel the deterioration of the olfactory abilities during the progression of the KD and the possible inflammatory mechanisms involved.

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# From Molecules to Noses: GC–MS and Canine Detection of Coronavirus VOC Signatures

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**Code:** 34

**Type of abstract:** TALK

**Keywords:** viral infection detection; scent detection dogs; volatile organic compounds; VOC; murine hepatitis virus; coronavirus; GC-MS; canine olfaction; machine learning classifier

Rapid and non-invasive detection of viral infections remains a major challenge in both public and animal health. Since viruses do not possess intrinsic metabolic activity, changes in the VOC profiles detected during infection, supposed to be attributed to host responses to infection , such as metabolic reprogramming, oxidative stress, and apoptosis. Volatile organic compounds (VOCs), produced as metabolic byproducts of viral replication, represent a promising source of diagnostic information that can potentially be detected through olfaction. The present study investigated whether trained scent detection dogs are able to discriminate virus-infected samples from uninfected controls based solely on odor cues, and whether these cues can be chemically characterized and computationally classified.

Using a biosafety level 2 murine coronavirus model (murine hepatitis virus strain 1, MHV-1), cell culture samples were prepared and presented to trained dogs under controlled conditions. Dogs were trained to distinguish infected samples from uninfected controls and their performance was evaluated in terms of sensitivity and response latency. In parallel, VOC profiles of infected and control samples were analyzed using gas chromatography–mass spectrometry (GC–MS).

Chemical analysis revealed distinct VOC patterns associated with viral infection. Fourteen VOCs were detected in infected samples and twelve in controls. Two compounds, 3-heptanone and 1-nonanol, were exclusively present in infected samples, while several others—including acetophenone, nonanal, decanal, and benzaldehyde—were significantly elevated in infected cultures. Behavioral testing demonstrated high detection sensitivity of trained dogs (0.95), exceeding responses to a previously trained reference odor (cinnamon; 0.88), with significantly shorter response latencies ($p = 0.04$). Effective detection required pooled sample volumes of approximately 600 µL, indicating a concentration-dependent threshold effect. Furthermore, a Random Forest classifier trained on GC–MS VOC profiles achieved a cross-validated accuracy of 0.82, supporting the discriminatory power of infection-related VOC signatures.

Together, these findings demonstrate that dogs can reliably detect viral infection-associated odor changes and suggest that quantitative VOC differences, rather than unique marker compounds alone, drive olfactory recognition. This study provides a robust experimental framework combining behavioral, chemical, and computational approaches, and highlights the potential of scent detection dogs as non-invasive biosensors for viral diagnostics in veterinary and public health applications.

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# Sexual dimorphism in the human olfactory bulb associated with age and Alzheimer's disease

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**Keywords:** Females, glia, males, neurodegeneration, stereology, volume

Alzheimer’s disease (AD) is the most prevalent neurodegenerative disorder and the leading cause of dementia worldwide [1]. Its incidence and prevalence are nearly twice as high in women as in men [2], yet biological sex remains underrepresented in AD research [3]. Neuropathologically, AD is characterized by the accumulation of aberrant proteins (amyloid-β and hyperphosphorylated tau), driving neurodegeneration and reactive gliosis [4]. These aggregates appear in the olfactory bulb (OB) from early disease stages, and olfactory dysfunction frequently precede clinical diagnosis [5]. This study is aimed to characterize sex-specific structural and neuroimmune alterations in the human OB during AD using unbiased stereological methods. Postmortem OB samples from 39 donors were classified as non-AD or AD and stratified by sex. OB volume was estimated using the Cavalieri method; neuronal density and total number with the Optical Fractionator; and glial reactivity with the Area Fraction Fractionator. Non-AD women showed higher basal microglial reactivity than non-AD men, indicating inherent sex differences in the OB neuroimmune milieu. Non-AD men had a higher total number of OB neurons than women, revealing baseline differences in neuronal composition. In AD, women exhibited reduced OB volume compared with men with AD, suggesting greater structural vulnerability. Conversely, men displayed a more pronounced neuronal loss pattern, with reduced neuronal density and total neuron number relative to non-AD men. Microglial activation was positively associated with age specifically in women with AD. These findings identify sexually dimorphic pathological signatures in the OB: women show higher basal neuroimmune reactivity and greater structural vulnerability, whereas men exhibit more marked neuronal depletion in AD. This study underscores the importance of incorporating sex-stratified approaches in neurodegenerative research. We thank the donors and the Spanish Biobank Network. Funded by UCLM/FUHNPAIIN (SBPLY/24/180225), UCLM/ERDF (2022-GRIN-34200), JCCM/ERDF (SBPLY/21/180501) and MICINN (PID2019-108659RB). Alonso-Gomez Andrea was supported by a UCLM/FSE+ predoctoral contract.

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# Temporal dynamics of the expression of amyloid-beta plaques and phospho-TAU neurofibrillary tangles in the olfactory and vomeronasal systems in the triple transgenic mice model of Alzheimer’s disease.

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Olfactory impairment is an early sign of Alzheimer’s disease observed both in patients and in animal models. However, the expression of the characteristic neuropathological markers (amyloid-beta plaques and phospho-TAU neurofibrillary tangles) has not been extensively characterized in the central olfactory structures in transgenic animal models. In addition, little information is available about the neuropathological hallmarks in the vomeronasal system. In the present study we aim to characterize the presence of beta-amyloid and phospho-TAU in the olfactory and vomeronasal systems in the triple transgenic Alzheimer's disease (3xTgAD) mouse model.

The analysis of the immunofluorescence for beta-amyloid at 3, 6, 9, 12 and 18 months, shows the evolution from the initial presence of intracellular beta-amyloid at some cortical, hippocampal and amygdaloid regions to widely distributed extracellular deposits of Aβ along the progression of Alzheimer's disease. TAU pathology appears restricted to the hippocampus at early ages (3-6 months old) and extends to cortical (mainly temporal) and amygdaloid regions at 12-18 months of age.

Notably, the main and accessory olfactory bulbs showed no pathological markers at any age. Regarding cortical olfactory structures, only the lateral entorhinal cortex showed beta-amyloid deposits in young animals, starting in some cases at 3 months-old. By 12 months some extracellular plaques were also present in this structure, which become more abundant in 18 months-old animals. A small number of plaques were also detected in the anterior olfactory nucleus in one sample at 12 months; and the number of plaques in this nucleus increased significantly at 18 months, when plaques were present in three of the six samples. Regarding the olfactory amygdala, intracellular beta-amyloid labeling was observed at 12 and 18 months in the nucleus of the lateral olfactory tract and the anterior and posterolateral cortical nuclei. With regard to the piriform cortex, in the 18 month-old animals, some extracellular plaques were observed in the most caudal sections in one of the samples. Finally, within olfactory structures TAU pathology was present only at the lateral entorhinal cortex in animals of 12 and 18 months of age.

In the vomeronasal system, neuropathological markers appeared only in the posteromedial cortical nucleus of the amygdala (PMCo). Both Aβ intracellular deposits and extracellular plaques were present in animals of 12 and 18 months of age, where also phospho-TAU labeling was observed. No pathological markers were present in the medial amygdala.

In summary, the characteristic neuropathological markers of Alzheimer’s disease appeared mainly in high-order cortical structures both in the olfactory (entorhinal cortex) and the vomeronasal (PMCo) systems. The olfactory system seems to be more sensitive to the pathology, since early Aβ deposition was present in the entorhinal cortex, whereas the vomeronasal system showed only late expression of either Aβ or phospho-TAU.

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# Density and morphology of doublecortin-ir neurons in the piriform cortex are affected in a sex-dependent way in a pre-symptomatic mouse model of Alzheimer’s disease

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**Keywords:** Olfaction, Alzheimer’s disease, neurogenesis, piriform cortex, doublecortin

Alzheimer’s disease (AD) is a neurodegenerative disorder characterized by loss of memory and other cognitive functions, with olfactory dysfunction being one of its prodromal symptoms. In mouse models of Alzheimer’s disease, this impairment is accompanied by deficits in postnatal neurogenesis in the ventricular-subventricular zone giving rise to new neurons in the olfactory bulbs. In the piriform cortex (Pir) there is a population of immature neurons of embryonic origin that slowly mature and integrate into the circuit as the animal ages. Since these immature neurons of the Pir have been shown to be affected by neurological diseases and environmental factors such as stress, and given the early nature of olfactory dysfunction in AD, we hypothesised that they might be altered in a mouse model of this disease. To test this hypothesis, we performed an immunofluorescence staining of immature neurons with the maker doublecortin (DCX) in coronal slices of the brain of 3-months old mice of both WT (B6/129) and pre-symptomatic 3xTg-AD mice of both sexes. Our results show that the density of DCX-ir neurons is decreased in transgenic mice in a sex-dependent way, with transgenic males being significantly affected. On the other hand, we have also studied the morphology of these immature neurons of the Pir with a three-component analysis (skeleton, Sholl analysis and fractal analysis), showing more significant morphological alterations in DCX neurons from transgenic males. In conclusion, this study suggests a sex-dependent impairment in the maturation of DCX neurons of the Pir during the pre-symptomatic period. Funded by Conselleria d’Educació, Cultura i Universitats from the Generalitat Valenciana (CIAICO/2023/027; CIAICO/2023/041) and the Spanish Ministry of Science, Innovation and Universities (PID2022-141733NB-I00; MCIU/ AEI /10.13039/501100011033/FEDER, UE).

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